Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

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Supplementary Appendix

Phase 1 Trials of rVSV EBOLA Vaccine in Africa and Europe

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Supplementary Appendix

Contents

Methods

VEBCON

In September 2014, the WHO brought together an African and European consortium (VEBCON, VSV EBola CONsortium) to harmonize parallel phase I trials of rVSV-ZEBOV and provide results crucial for phase II/III trials relevant to the current outbreak of Ebola virus disease (EVD). VEBCON centers include Lambaréné (Gabon), Kilifi (Kenya), Hamburg (Germany) and Geneva (Switzerland), with laboratory support in Marburg (Germany), London (UK) and Geneva (Switzerland). Its members are: Selidji Todagbe Agnandji (Centre de Recherches Medicales de Lambaréné, Gabon, Institut für Tropenmedizin, Universitätsklinikum Tübingen, Germany) and Sanjeev Krishna (St George's University of London, UK, Institut für Tropenmedizin, Universitätsklinikum Tübingen, Germany, Centre de Recherches Medicales de Lambaréné Lambarene, Gabon); Peter G. Kremsner and Jessica S. Brosnahan (Institut für Tropenmedizin, Universitätsklinikum Tübingen, Germany, Centre de Recherches Medicales de Lambaréné, Gabon); Philip Bejon and Patricia Njuguna (Kenya Medical Research Institute, Kilifi, Kenya); Marylyn M. Addo (University Medical Center Hamburg, Germany); Stephan Becker and Verena Krähling (Institute of Virology, Marburg, Germany); Claire-Anne Siegrist and Angela Huttner (Geneva University Hospitals); Marie-Paule Kieny, Vasee Moorthy, Patricia Fast, Barbara Savarese, Olivier Lapujade (World Health Organization, Geneva, Switzerland).

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Study Timelines

Regulatory and ethical approvals were granted by November 7th and 4th in Germany, by 31th October and 30th of October in Gabon, by 18th November and 8th December in Kenya and by November 3rd in

Switzerland. Immunizations were initiated on November 10th, November 21st, December 17th and November 10th respectively. All VEBCON studies were reviewed and approved by the WHO's ethics committee (EC). Vaccination cycles ended in Germany on December 9th, in Switzerland on December 9th (safety-driven study hold) and in Gabon on January 9th. The last immunization of subjects included in this report took place on January 19th, 2015, in Kenya.

Dose Escalation, Randomization and Blinding

For the dose-escalation trials, the first subjects in each site receiving a new dose were vaccinated at least 48h (Hamburg, Lambaréné) or 72h (Kilifi) before further vaccinations (see study protocols). Dose escalation occurred upon review of safety data of the first 5-10 vaccinees of the lowest dose by the DSMB (Lambaréné, Kilifi), or Local Safety Board (Hamburg).

For the Geneva randomized controlled trial (RCT), two randomization schemes were developed: deployable subjects were randomized 1:1 to rVSV-ZEBOV at 10^7 or 5 x 10^7 pfu, and non-deployable subjects 1:1:1 to either dose of vaccine or placebo. Not including a placebo arm for deployable volunteers at risk of subsequent exposure to ebolavirus was a specific request of WHO and other international institutions (Doctors without Borders) based in Geneva. A statistician not involved in the study analysis generated the two randomization lists at www.randomization.com using investigator-blinded, randomly permuted blocks of varying sizes prior to study launch. Only the pharmacist, an infectious disease physician not involved in the study, and a laboratory technician have copies of the lists.

The Geneva trial was double blind. Clinical staff and study participants were unaware of subjects' treatment allocation (whether higher- or lower-dose vaccine for deployables, and whether vaccine at either dose or placebo for non-deployables). Through sample coding, specialized laboratory staff performing specific assessments were blinded to the treatment administered. Through recoding of subject numbers (performed by secuTrial, Berlin, Germany), data analysts remain blinded to individual

treatment allocations. Given the uneven number of subjects in each treatment arm, the study statistician (CC) was not blinded to group treatment allocation during analyses. Study investigators and one non-deployable subject with arthritis became aware of his allocation to vaccine as opposed to placebo when rVSV was detected in his synovial fluid (case #9). The blind was intentionally lifted on February 26, 2015 (after completion of the day 84 [D84] visit) for the 11 subjects with arthritis, and on April 7, 2015 (after the D84 visits) for all remaining subjects randomized before the study hold.

Vaccine Reconstitution

rVSV–ZEBOV was formulated with 2.5 g/L recombinant human serum albumin and 10mM Tris (pH 7.2) and dispensed in a 1.0 ml unit dose vial as $1x10^8$ pfu/ml, with the same batch used at all VEBCON trial sites.

Vaccine Reconstitution in Gabon

Preparation of $3x10^6$ pfu/ml and $3x10^5$ pfu/ml vaccine: 1 ml vial (formulated at $1x10^8$ pfu/ml) was diluted with 9 ml of 0.9% sodium chloride diluent. From this, 3.5 ml were added to 7.5 ml of saline diluent to obtain 11 ml of BPSC1001 at $3x10^6$ pfu/ml. From the previous preparation 1 ml was added to 9 ml of 0.9% sodium chloride diluent to obtain 10 ml at $3x10^5$ pfu/ml.

Vaccine Reconstitution in Kenya and Germany

Preparation of $3x10^6$ pfu/ml vaccine: 0.31 ml of vaccine (formulated at $1x10^8$ pfu/ml) was added to 10 ml of normal saline under sterile conditions creating a final volume of 10.31 ml of virus concentration of $3x10^6$ pfu/ml. 1 ml was used for vaccination.

Preparation of $2x10^7$ pfu/ml vaccine: The content of one vial (formulated at $1x10^8$ pfu/ml) of virus was added to a sterile syringe containing 4 ml of normal saline under sterile conditions creating a final volume of 5 ml at a concentration of $2x10^7$ pfu/ml. 1 ml was used for vaccination.

Preparation of 2x10^7 pfu/ml vaccine: 1 ml vial of 10^8 pfu/ml was diluted with 4 ml of 0.9% sodium chloride diluent to obtain 5 ml at 2x10^7 pfu/ml.

Vaccine Reconstitution in Geneva

The 5x10^7 pfu dose was achieved by adjusting the injection volume to 0.5 ml. The 10^7 pfu dose was prepared by mixing 0.2 ml of vaccine solution (10^8 pfu/ml) with 0.8 ml of normal saline (NS) and harvesting 10^7pfu/0.5 ml. Placebo syringes contained 0.5 ml NS and were packaged identically.

Specific Clinical Evaluation

Work-up for Subjects identified with Arthralgia or Skin Lesions in Geneva

Arthrocentesis was performed in only one case, as sufficient fluid was at hand and informed consent granted. Determination of acute-phase reactants included complete blood counts, C-reactive protein levels and erythrocyte sedimentation rates. Subjects underwent HLA-B27 testing. Autoantibody screens (rheumatoid factor [RF], anti-citrullinated antibodies [anti-CCP], antinuclear, anti-Sm, anti-n-RNP, anti-SSA [Ro], anti-SSB [La], anti-Scl-70 and anti-Jo-1) with measurement of total complement activity (CH50) and C3, C4, and serology for parvovirus B19, *Mycoplasma pneumoniae*, and *Chlamydia trachomatis* were performed at time of diagnosis and compared to baseline samples, if positive. All subjects were assessed for the functional impact of the joint manifestation (RAPID3 score)[1] and their inflammatory disease activity (DAS44).[2] Ultrasound and/or magnetic resonance imaging (MRI) was performed by accredited staff of the rheumatology/imaging division, using standard techniques and appropriate controls including unaffected joints.

One papular and two vesicular skin lesions (from a total of three individuals) were biopsied and assessed histologically. rVSV viremia was assessed at time of joint/skin symptoms, and was consistently undetectable.

rVSV Viral Load Detection Assay

rVSV viral loads were monitored in body fluids with VSV-specific one-step Reverse Transcriptase TaqMan® quantitative polymerase chain reaction (RT TaqMan qPCR) from day 0 until day 28. The estimated copy number of our target gene VSV nucleoprotein (VSV-NP) represents the copy number of the vaccine. To assess rVSV viral loads of Lambaréné and Kilifi participants, samples were shipped to London (St. George's University of London), whereas Hamburg and Geneva monitored their samples directly at their trial centers.

Participants in Hamburg were screened for viremia and viral shedding in urine and saliva daily in real time through day 7 as well as on days 14 and 28 per request of the respective competent authority and study subjects remained hospitalized until plasma viremia was cleared. Lambaréné and Kilifi monitored viremia/shedding on days 0, 1, 2 and 7 and 0, 1, 3 and 7, respectively. Geneva analyzed samples on days 0, 1, 3±1 and 7±2 (or > day 7 if viremia was still detectable on day 7 or in subjects with arthritis and/or skin lesions). Each RT qPCR run included standards as well as no-template controls. Measurements of controls and samples from vaccinees were analyzed in duplicate.

In Hamburg, total ribonucleic acid (RNA) from plasma and urine specimens and saliva swabs (Viral Transport Kit, BD) was extracted using the QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany) according to manufacturer's instructions. RT qPCR was performed using the AgPath-ID™ One-Step RT-PCR Kit (Ambion, CA, USA) as described by Günther et al.[3] In this assay, VSV-specific primers target the VSV-NP gene (VSV-forward: GACCTTGTATCCTTG AAAGCC; VSV-reverse: CATTTGTGTTCTGCCCACTC; VSV probe FAM-TGCTTCCAGAA CCAGCGCAGATGACAAA-BHQ1). One-step RT-qPCR was performed on the Rotor-Gene Q cycler (Qiagen, Hilden, Germany) with following protocol: one cycle at 50°C for 15 minutes, one cycle at 95°C for 10 minutes followed by 45 cycles, each at 95°C for 10 seconds and at 60°C for 40 seconds. Data were analyzed via Rotor-Gene Q Series Software.

Samples from Lambaréné and Kilifi were stored in Trizol LS Reagent (Life Technologies, CA, USA) and transported to St George's University of London for PCR analysis. Total RNA was extracted using the

phenol-chloroform method followed by viral RNA Mini Kit (Life Technologies) according to manufacturer´s instructions. Each RNA extraction included a no-template and a positive control. Geneva extracted RNA (with spiked Canine Distemper Virus (CDV) as internal control) using Nuclisens EasyMag (BioMérieux, Geneva, Switzerland).

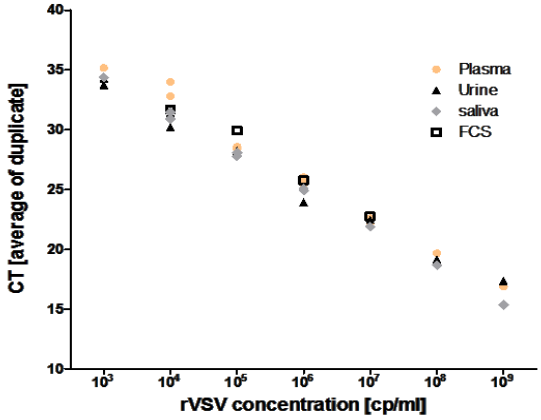
London and Geneva performed RT TaqMan® qPCR using QuantiTect Probe RT-PCR Kit (Qiagen) and Uracil DNA Glycosylase (Roche Diagnostics) targeting the VSV-NP gene (VSVNP-F 5'-CGGAGGATTGACGACTAATGC-3', VSVNP-R 5'-CGAGCCATTC GACCACATC-3', VSV-NP Probe 5'-FAM-CGCCACAAGGCAG-MGBNFQ-3'). RT-qPCR was performed on the CFX96 (Bio-Rad) or on the StepOne System Plus (Life Technology) in London and Geneva, respectively; and data were analyzed via the respective software. Both sites used the following PCR protocol: 10 minutes at 15ºC, 30 minutes at 50ºC, 15 minutes at 95ºC followed by 45 cycles of 15 seconds at 94ºC and 1 minute at 60ºC. Each RT-qPCR included a no-template control, a no-reverse-transcriptase control and a standard curve of three RNA concentrations.

Quantification of rVSV Standard

Quantification Performed in Hamburg

To generate calibration curves, we used a serial 10-fold dilution of rVSV-ZEBOV with a maximum range of 6-log. Calibration curves were processed under the same RT-qPCR reaction conditions as the test samples. To ensure stability and reproducibility of our assay, we spiked the vaccine into the primary samples plasma, urine and saliva of healthy unvaccinated individuals. This was repeated independently. Furthermore, FCS was used as a positive control to investigate potential inhibition in primary samples. As shown in Figure S1, we validated the reproducibility and stability of both RNA extraction and PCR assay and detected only a low degree of inhibition in plasma samples.

Figure S1. Validation of reproducibility and stability of RNA extraction and PCR efficiency. Serial dilution of the vaccine was spiked into different primary samples. Different ranges were tested. A no-spike-in control was included and showed no PCR signal (data not shown).

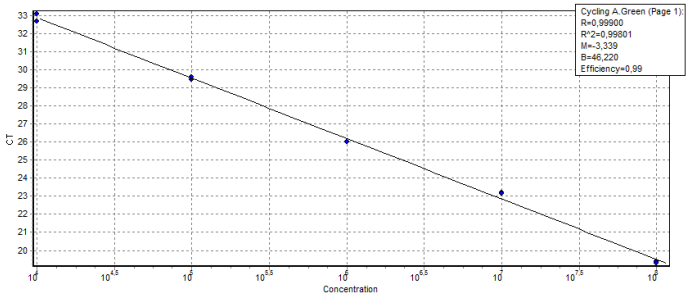


We received PCR signals over the entire concentration range. Samples with a mean threshold cycle (Ct) value of < 35 were considered positive. Linearity was only given between $1x10^8$ copies/ml to $1x10^3$ copies/ml, which corresponds to $2x10^7$ and 20 copies per reaction, respectively.

Based on the calibration curves, the data were subdivided into three groups; first, quantifiable copies/ml; second, copies/ml below the limit of quantification (BLQ) and third, samples with no positive PCR signal, thus not detectable and referred to as below the limit of detection (BLD). Hence, we distinguished between limit of detection and quantification. Samples with <200 copies/ml were grouped into BLQ. Their viral load was estimated as 50 copies/ml.

A linear regression was plotted, constituting the standard curve and providing PCR efficiency.

Figure S2. PCR Efficiency. Calibration curve of spiked saliva (concentration: $1x10^8$ to $1x10^3$ copies/ml) had a PCR efficiency of 0.99.



Quantification Performed in Geneva and London

rVSV-ZEBOV RNA was extracted from the vaccine in Geneva and supplied to London at a concentration of $2x10^8$ copies/ml to carry out calibration curves. The vaccine was diluted in BaseMatrix (Ruwag, Switzerland) in Geneva, and DEPC-treated H_2O in London, to achieve concentrations ranging from $8x10^7$ to 80 copies/ml of RNA. Detection of RNA was linear down to 800 copies/ml. As there was an 8-

fold concentration of samples (from 200 µl plasma from volunteers to 25 µl eluate containing RNA), samples with ≥100 copies/ml RNA were considered to be detectable and quantifiable. Samples between 30-100 copies/ml RNA were considered to be detectable but not quantifiable.

In Geneva, the limit of detection (LOD) was defined as 30 copies/ml. Samples with values below this threshold were arbitrarily given a value of 15 copies/ml. The assay was linear for quantification from 100 to 10^7 copies/ml. The limit of quantification in plasma was therefore set at 100 copies/ml. Samples between 30 and 100 copies/ml were arbitrarily given a value of 65 copies/ml. The interassay coefficient of variation in Geneva was 1.2% (mean 4.02 log copies/ml, SD 0.05) among the 32 experiments performed.

The calibration curves were processed under the same RT-qPCR conditions as the test samples.

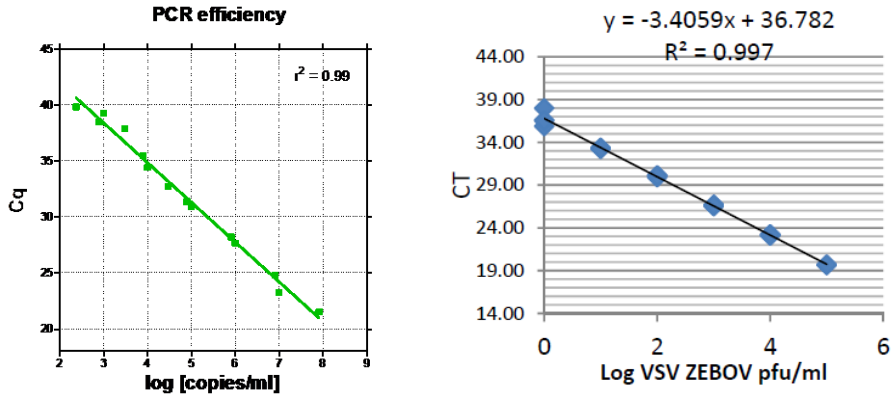


Figure S3. Quantification performed in London (left panel) and Geneva (right panel). A linear regression (r^2 = 0.99) was plotted.

Comparison of qPCR Assays from the Different Sites

Samples from Lambaréné and Kilifi were analyzed for viremia/shedding via RT qPCR in London, whereas Hamburg and Geneva directly monitored samples at their trial centers. As Hamburg and Geneva/London performed RT-qPCR with different assays, a direct comparison was required to compare both data sets.

For this approach, standards and samples from vaccinees were shipped from London to Hamburg. Chosen samples were blinded and covered a range of concentrations including (1) quantifiable, (2) not quantifiable and (3) not detectable.

Results are listed in Table S1. In column 2, results from London are listed. Column 3 represents data generated in Hamburg, where CT values were related to London's standard curve. In column 4, results are listed, where CT values were related to Hamburg's standard curve. We observed little variation in copies/ml. Quantifiable copies/ml showed a maximum variance of 2.6-fold.

Table S1. Comparison of qPCR assays between sites.

Sample n°	Copies/ml (Analyzed in London)		Copies/ml (Analyzed in Hamburg with London's standard curve)		Copies/ml (Analyzed in Hamburg with Hamburg's standard curve)	
	Copies/ml	Category	Copies/ml	Category	Copies/ml	Category
1	8	BLD	0	BLD	0	BLD
2	730	730	539	539	930	930
3	1761	1761	823	823	1505	1505
4	54	BLQ	26.2	BLQ	31	BLQ
5	454	454	153	BLQ	177	BLQ
6	1412	1412	1216	1216	2400	2400
7	16	BLD	64	BLQ	116	BLQ
8	59	BLQ	50.7	BLQ	111	BLQ

BLD: Below limit of detection

BLQ: Below limit of quantification

Cell Culture

The Vero 1008 C (E6) cell line was originally obtained from ATCC (Maryland, USA) and cultured in Gibco® Dulbelcco's modified Eaggle's medium (DMEM) with GlutaMAX™, high glucose and HEPES supplemented with 10% fetal bovine serum (FBS, Euro-Clone), 1% Gibco® penicillin/streptomycin solution (5,000 U/mL) and 1x Gibco® MEM non-essential amino acids solution (100x) at 37°C, 5%CO_2.

rVSV-ZEBOV Replication Assessment

rVSV-ZEBOV is an attenuated replication-competent virus with a cytolytic life cycle. We therefore used the virus induced cytopathic effect to assess if replication competent rVSV-ZEBOV were present in

clinical samples (swabs). Vero E6 cells were seeded in 24 wells plates (Nunc™) 16-20 hours prior to replication assessment. The samples (0.2 ml per well) were adsorbed to cells monolayers for 1 hour at 37°C, 5% CO_2. After adsorption, 0.8 ml of cell medium were added to each well and 0.4 ml of the diluted inoculums were collected and stored at -80°C (T0). The virus-induced cytopathic effect was monitored by inverted microscopy (Nikon Eclipse Ti-5) after 48 hours of incubation at 37°C and 5%CO_2. 0.4 ml of the 48 hours culture media were harvested and stored at -80°C (T48). rVSV-ZEBOV was included as positive control and the detection threshold defined at 2 (transport medium) or ≥ 10^2 (plasma) pfu/ml. T0 and T48 samples were processed for VSV specific RT-PCR, which confirmed that the observed cytopathic effect was consistent with rVSV-ZEBOV replication. All investigated swabs were collected in 3 ml virus transport medium (Copan) and systematically treated with a mix of antibiotics (equal volume mix: penicillin/streptomycin solution (5,000 U/mL), gentamicin (10 mg/ml), Fungizone®, 1 mix drop/ml of virus transport medium, Gibco®) for 10 minutes prior to adoption to cells.

For rVSV-ZEBOV replication assessment by immunofluorescence, Vero E6 cells were allowed to attach for 8-10 hours, in 24 well plates (Nunc™) containing cell culture-treated glass slides. After 48h, cell culture supernatants recovered from an infectious swab (0.2 ml per well) were adsorbed to cell monolayers for 1h at 37°C, 5%CO_2. After adsorption, 0.8 ml of cell medium was added to each well. After 20h incubation at 37°C and 5%CO_2, the virus-induced cytopathic effect was monitored and the cells were stained for viral antigens.

Detection of the VSV Matrix Protein (M) by Immunofluorescence

Infected cells cultured on glass slides were washed twice with Dulbecco's Phosphate-Buffered Saline (DPBS, Gibco®) and fixed with a 4% paraformaldehyde-PBS solution for 15 minutes at 37°C. Fixed cells were washed twice with DPBS and incubated with a 1% FCS-DPBS solution for 15 minutes at room temperature (RT), followed by 15 minutes permeabilization (1x BD Perm/Wash™ solution) at RT.

Permeabilized cells were stained for VSV M using a mouse anti-VSV matrix monoclonal antibody (23H12, laboratory of Prof. Douglas S. Lyles, Wake Forest School of Medicine) kindly provided by Prof. Stefan Kunz (Institute of Microbiology of Lausanne, Switzerland) and Prof. Juan Carlos de la Torre (The Scripps Research Institute, La Jolla, California), combined with a goat anti-mouse IgG antibody FITC containing 0.02% Evans Blue counterstain (LIGHT DIAGNOSTICS™, Merck Millipore, Schaffhausen, Switzerland). Antibodies were diluted in 1x BD Perm/Wash™ solution. Fluorescence was monitored using a Nikon eclipse E600 microscope with a Nikon digital sightscreen.

All experiments involving infectious/non-inactivated rVSV-ZEBOV were performed in an rVSV-ZEBOV dedicated PSM II cabinet and cell culture incubator within the biosafety level 2 laboratories at the Virology Laboratory of the Geneva University Hospital.

Immunogenicity

ANTI-GP ELISA Assay (USAMRIID)

EBOV GP-antibodies were measured by ELISA using the homologous Zaire-Kikwit strain glycoprotein (GP) as antigen following USAMRIID SOP AP-03-35-00. Briefly, serum samples were added to 96-well microtiter plates pre-coated with recombinant GP Zaire Kikwit and allowed to react. A reference standard, plus positive and negative controls were also included. EBOV GP-specific antibodies were detected by the addition of an anti-human secondary immunoglobulin G (IgG) antibody conjugated to horse-radish peroxidase, followed by a colorimetric substrate. Optical density (OD) was measured at an absorbance wavelength of 450 nm. The relative amount of EBOV GP-specific antibody in a given serum sample was calculated by (i) interpolation from the reference standard curve using a 4-parameter logistic model and reported as end-point titers calculations expressed as the reciprocal of the highest serum dilution yielding an OD reading greater than the cut-off OD of 0.2.

Whole Virion ELISA Assay (Marburg)

EBOV (Zaire Ebola Virus, Guinea isolate, C7, AccNo: KJ660347) and mock antigens were prepared from cell culture supernatants of EBOV-infected (6 d post infection) or uninfected (6 d post seeding) VeroE6 cells. Concentration of particles from the supernatant was performed via ultracentrifugation through a 20% sucrose cushion at 4°C and 76,000 x g for 2 hours. Resulting pellets were resuspended in PBS containing 1% SDS. To inactivate EBOV, samples were boiled for 10 minutes before they were removed from BSL-4 facility of the Philipps University of Marburg. High binding single-break strip microtiter plates (Greiner bio-one, Cat.No. 705073 & 705075) were coated with 50 µl EBOV or mock antigen (both diluted 1:1000 in PBS, final protein concentration of viral antigen about 2 µg/ml) and incubated for 16-17 hours at 4°C. Further incubations were performed at room temperature. ELISA plates were washed three times with PBS/0.1% Tween®20 (PBST, Sigma Aldrich, P7949) and then blocked for 45 minutes with PBS containing 5% milk powder. Washing procedure was repeated three times with PBST. Sera of volunteers were diluted 1:200 in PBST containing 1% milk powder and allowed to react with EBOV and mock antigen for 1 hour. After washing the plates for three times with PBST, polyclonal rabbit anti-human IgG/HRP (DAKO, P0214) was used for detection. Plates were washed two times with PBST and two times with PBS. Bound IgG was detected by SureBlue™ TMB Microwell Peroxidase substrate (KPL, 52-00-00). TMB Stop Solution (KPL, 50-85-04) was added after 10 minutes and optical density was determined at 450 nm – 620 nm. A panel of positive and negative controls and standards was run each time the assay was performed to validate the experiment. Each sample was analyzed in duplicate and corrected OD values were calculated by subtracting mock OD values from values obtained by incubating the same serum with EBOV antigen. To calculate the fold induction of antibody responses between D0 and D28, corrected OD values of each volunteer were compared. A corrected OD value of negative control + 10% was set as the cut-off. Only if the D28 OD value was above this cut off was x-fold induction calculated; otherwise induction was set to 1.

Pseudovirion Neutralization Assay (PsVNA, USAMRIID)

EBOV pseudovirions (PsV) were prepared using recombinant vesicular stomatitis virus (VSV) ΔG with luciferase reporter (VSV*rLuc) by methods similar to those described previously.[4,5] Ebola GP used in pseudotyping was provided by a plasmid expressing the EBOV Zaire 95 Kikwit GP, pWRG7077 EBOVco[6]. The PsVNA was performed essentially as previously described.[7] Briefly, an initial 1:10 dilution (in triplicate) of heat-inactivated sera was made followed by five-fold serial dilutions that were mixed with an equal volume of complete EMEM containing EBOV PsV at approximately 4,000 focus-forming units (FFU) per well of a 96-well plate, and 10% (v/v) human complement (Sigma). This mixture, containing 5% human complement, was incubated overnight at 4°C. Following this incubation, 50 µl of the PsV + antibody mixture were inoculated onto Vero cell monolayers in a clear bottom black-walled 96-well plate (Corning) and incubated at 37°C for 18-24 hr. Cells were lysed according to the luciferase kit protocol (Promega). A Tecan M200 Pro microplate reader was used to acquire flash luciferase data. The raw data (relative light unit values) were exported to GraphPad Prism version 6.04, where the % neutralization data were normalized to the untreated PsV signal. % neutralization data were fit to a four-parameter logistic equation using GraphPad Prism and then PsVNA 50% (PsVNA50) neutralization titers were interpolated from the curves for each sample. Geometric mean titers (GMT) for replicates are reported.

Neutralization Assay (Ebola Virus, Marburg)

Volunteer blood sera were incubated at 56°C for 30 min for complement inactivation. After centrifugation at 13,000 rpm for 10 min, sera were serially diluted starting from 2^3 to 2^{10} in Dulbecco's modified Eagle's medium (DMEM, Gibco) supplemented with 2% fetal calf serum (FCS, Gibco), penicillin (100 U/ml), streptomycin (100 mg/ml), and L-glutamine (2 mmol/l) (all from Invitrogen) in 96 well culture plates. 100 $TCID_{50}$ units of Ebola virus (Zaire, isolate Mayinga, AF086833) were added to

the serum dilutions. Following incubation at 37°C for one hour, Vero cell suspension in DMEM containing 2% FCS was added. Plates were then incubated at 37°C with 5% CO_2 and cytopathic effects (CPE) were evaluated at seven days post infection. Neutralization titers were calculated as GMT of four replicates. Cut-off was defined as GMT plus standard deviation (SD) of all volunteers of one cohort at D0. Neutralization assays were performed in the BSL-4 laboratory of the Institute of Virology, Philipps-University Marburg, Germany.

Western Blot Analysis

ZEBOV C7 antigen (5 µl/ blot) was separated on 10% SDS PAGE and transferred to PVDF membranes. Membrane was cut into strips and stained with respective human sera at a dilution of 1:100 and an anti-human rabbit peroxidase (POD)-coupled secondary antibody (1:40,000). As positive control an anti-ZEBOV goat serum (1:30,000) and donkey anti-goat secondary antibody (1:40,000) were used.

Data Analysis

Description of Antibody Titers

Antibody titers were described by geometric means (logarithm base 10) and 95% confidence intervals. Antibody titers measured at day 0, 28 or 6 months were compared with Wilcoxon's test for paired data. Seropositivity rates at days 0, 28 and 6 months were reported and compared with the McNemar test. For each center, titers, seropositivity and seroresponse rates were compared between dose groups by Mann-Whitney and Fisher's exact tests. These analyses were conducted according to both intention-to-treat and per protocol principles.

Association Between Doses and GMT/GMC, Seropositivity and Seroresponse Rates

Association between doses and GMT/geometric mean concentrations (GMC) were explored with linear mixed effects regression with a random intercept to capture between-center variability. The Cochran-Armitage test was used to test trends between seropositivity (and seroresponse) rates and doses. These analyses were conducted according to both intention-to-treat and per protocol principles.

Correlations Among Antibody Titers

Strength of associations between variables was assessed by Spearman's rank correlation. These analyses were conducted according to both intention-to-treat and per protocol principles.

Results

Clinical Characteristics at Baseline

Table S2 indicates baseline values for all study participants.

Table S2: Clinical characteristics at baseline.

	Hamburg		Lambaréné		Kilifi		Geneva			All subjects
	$3x10^6$ pfu (n=10)	$2x10^7$ pfu (n=10)	$3x10^5$ pfu (n=20)	$3x10^6$ pfu (n=19)	$3x10^6$ pfu (n=20)	$2x10^7$ pfu (n=20)	$1x10^7$ pfu (n=35)	5×10^7 pfu (n=16)	Placebo (n=8)	(n=158)
Biological Characteristics at Baseline										
Hemoglobin, g/L	147 (19)	151 (12)	135 (19)	138 (12)	143 (15)	146 (12)	143 (14)	144 (11)	142 (11)	**143 (15)**
Platelets, G/L	247 (57)	236 (59)	203 (56)	220 (48)	246 (67)	255 (45)	246 (48)	235 (45)	226 (56)	**235 (56)**
Leucocytes, G/L	7.5 (2)	6.9 (2)	6.3 (1.5)	6.7 (1.7)	5.6 (1.1)	5.0 (1.3)	5.9 (1.2)	6.6 (2.1)	5.7 (1.6)	**6.2 (1.7)**
Lymphocytes, G/L	1.8 (0.4)	1.9 (0.9)	2.3 (0.7)	2.3 (0.6)	2.5 (0.5)	2.2 (0.6)	2.0 (0.5)	2.0 (0.5)	2.0 (0.7)	**2.1 (0.6)**
Neutrophils, G/L	4.9 (1.8)	4.3 (1.2)	2.7 (1.1)	2.6 (0.7)	2.8 (1.0)	2.4 (0.8)	3.3 (0.1)	3.8 (1.8)	3.1 (1.1)	**3.1 (1.3)**
Monocytes, G/L	0.5 (0.1)	0.5 (0.3)	0.5 (0.2)	0.5 (0.1)	0.2 (0.1)	0.2 (0.1)	0.5 (0.2)	0.6 (0.2)	0.5 (0.1)	**0.4 (0.2)**
Creatinine (mg/dL)	0.9 (0.1)	0.9 (0.2)	0.8 (0.2)	0.8 (0.1)	1.1 (0.1)	1.0 (0.1)	0.9 (0.1)	0.9 (0.2)	0.9 (0.2)	**0.9 (0.2)**
AST (U/L)	19 (12)	17 (4)	23 (3)	22 (3)	ND	ND	15 (5)	15 (7)	16 (6)	**18 (7)**
ALT (U/L)	22 (15)	28 (14)	15 (6)	16 (7)	21 (9)	28 (13)	21 (15)	16 (6)	21 (15)	**21 (12)**

Values are expressed as mean (standard deviation), unless otherwise specified.

ND = Not determined.

Acute reactogenicity

Table S3 indicates adverse events reported by all study participants within 14 days of injection.

Table S3: Adverse Events within 14 Days after Receipt of rVSV-ZEBOV Vaccine or Placebo*.

Event		Hamburg		Lambaréné		Kilifi	Geneva		
		$3x10^6$ pfu (n=10)	$2x10^7$ pfu (n=10)	$3x10^5$ pfu (n=20)	$3x10^6$ pfu (n=19)	$3x10^6$ pfu (n=20)	$1x10^7$ pfu (n=35)	$5x10^7$ pfu (n=16)	Placebo (n=8)
Any Adverse Event	None	0 (0%)	1 (10%)	5 (25%)	2 (11%)	3 (15%)	1 (3%)	0 (0%)	2 (25%)
	Mild	5 (50%)	5 (50%)	12 (60%)	9 (47%)	8 (40%)	11 (31%)	3 (19%)	5 (63%)
	Moderate	4 (40%)	3 (30%)	3 (15%)	8 (42%)	6 (30%)	14 (40%)	10 (63%)	1 (13%)
	Severe	1 (10%)	1 (10%)	0 (0%)	0 (0%)	3 (15%)	9 (26%)	3 (19%)	0 (0%)
Solicited Local Symptoms									
Erythema	None	9 (90%)	8 (80%)	20 (100%)	19 (100%)	20 (100%)	35 (100%)	15 (94%)	8 (100%)
	Mild	1 (10%)	2 (20%)	-	-	-	-	1 (6%)	-
Swelling / induration	None	9 (90%)	9 (90%)	20 (100%)	19 (100%)	19 (95%)	34 (97%)	16 (100%)	8 (100%)
	Mild	1 (10%)	1 (10%)	-	-	1 (5%)	1 (3%)	-	-
Pain	None	4 (40%)	5 (50%)	18 (90%)	11 (58%)	10 (50%)	9 (26%)	3 (19%)	8 (100%)
	Mild	6 (60%)	5 (50%)	2 (10%)	8 (42%)	8 (40%)	26 (74%)	12 (75%)	-
	Moderate	-	-	-	-	2 (10%)	-	1 (6%)	-
Solicited Systemic Symptoms									
Objective fever	None	8 (80%)	7 (70%)	20 (100%)	17 (90%)	14 (70%)	26 (74%)	12 (75%)	8 (100%)
	Mild	2 (20%)	3 (30%)	-	1 (5%)	6 (30%)	9 (26%)	4 (25%)	-
	Moderate	-	-	-	1 (5%)	-	-	-	-
Subjective fever	None	9 (90%)	10 (100%)	19 (95%)	12 (63%)	17 (85%)	13 (37%)	6 (38%)	7 (88%)
	Mild	1 (10%)	-	1 (5%)	4 (21%)	3 (15%)	14 (40%)	5 (31%)	1 (13%)
	Moderate	-	-	-	3 (16%)	-	6 (17%)	4 (25%)	-
	Severe	-	-	-	-	-	2 (6%)	1 (6%)	-
Chills	None	7 (70%)	7 (70%)	20 (100%)	17 (89%)	17 (85%)	18 (51%)	6 (38%)	8 (100%)
	Mild	3 (30%)	3 (30%)	-	2 (11%)	1 (5%)	7 (20%)	4(25%)	-
	Moderate	-	-	-	-	1 (5%)	7 (20%)	5 (31%)	-
	Severe	-	-	-	-	1 (5%)	3 (9%)	1 (6%)	-
Myalgia	None	2 (20%)	4 (40%)	20 (100%)	13 (68%)	16 (80%)	12 (34%)	5 (31%)	6 (75%)
	Mild	5 (50%)	6 (60%)	-	3 (16%)	3 (15%)	15 (43%)	6 (38%)	1 (13%)
	Moderate	3 (30%)	-	-	3 (16%)	1 (5%)	5 (14%)	5 (31%)	1 (13%)
	Severe	-	-	-	-	-	3 (9%)	-	-

* Percentages may not total 100 because of rounding.

Table S3 (cont): Adverse Events within 14 Days after Receipt of rVSV-ZEBOV Vaccine or Placebo*

Event		Hamburg		Lambaréné		Kilifi	Geneva		
		$3x10^6$ pfu (n=10)	$2x10^7$ pfu (n=10)	$3x10^5$ pfu (n=20)	$3x10^6$ pfu (n=19)	$3x10^6$ pfu (n=20)	$1x10^7$ pfu (n=35)	$5x10^7$ pfu (n=16)	Placebo (n=8)
Headache	None	3 (30%)	5 (50%)	16 (80%)	10 (53%)	11 (55%)	14 (40%)	8 (50%)	5 (63%)
	Mild	6 (60%)	4 (40%)	3 (15%)	6 (32%)	6 (30%)	11 (31%)	4 (25%)	3 (38%)
	Moderate	1 (10%)	1 (10%)	1 (5%)	3 (16%)	2 (10%)	10 (27%)	3 (19%)	-
	Severe	-	-	-	-	1 (5%)	-	1 (6%)	-
Fatigue	None	6 (60%)	9 (90%)	10 (50%)	10 (53%)	19 (95%)	13 (37%)	8 (50%)	6 (75%)
	Mild	3 (30%)	1 (10%)	7 (35%)	5 (26%)	1 (5%)	5 (14%)	5 (31%)	2 (25%)
	Moderate	-	-	3 (15%)	4 (21%)	-	16 (46%)	3 (19%)	-
	Severe	1 (10%)	-	-	-	-	1 (3%)	-	-
Gastro-intestinal symptoms	None	10 (100%)	8 (80%)	15 (75%)	15 (79%)	17 (85%)	26 (74%)	12 (75%)	8 (100%)
	Mild	-	1 (10%)	5 (25%)	3 (16%)	3 (15%)	5 (14%)	4 (25%)	-
	Moderate	-	1 (10%)	-	1 (5%)	-	3 (9%)	-	-
	Severe	-	-	-	-	-	1 (3%)	-	-
Unsolicited Adverse Events									
Oral vesicle	None	8 (80%)	9 (90%)	20 (100%)	19 (100%)	20 (100%)	35 (100%)	16 (100%)	8 (100%)
	Mild	2 (20%)	1 (10%)	-	-	-	-	-	-
Arthralgia#	None	10 (100%)	9 (90%)	20 (100%)	12 (63%)	16 (80%)	30 (86%)	14 (88%)	8 (100%)
	Mild	-	-	-	4 (21%)	-	3 (9%)	2 (13%)	-
	Moderate	-	1 (10%)	-	3 (16%)	4 (20%)	2 (6%)	-	-
Arthritis$	None	9 (90%)	10 (100%)	20 (100%)	19 (100%)	19 (95%)	27 (77%)*	13 (81%)	8 (100%)
	Mild	-	-	-	-	-	4 (11%)	-	-
	Moderate	1 (10%)	-	-	-	1 (5%)	-	1 (6%)	-
	Severe	-	-	-	-	-	4 (11%)	2 (13%)	-

* Percentages may not total 100 because of rounding.

#Arthralgia was observed during the first week after immunization.

$Arthritis was observed during the second week after immunization.

Blood Counts per Day and Dose Group
Safety laboratory tests were performed on screening days and follow-up time visits.

Table S4 lists the mean blood count values.

Event		Hamburg		Lambaréné		Kilifi		Geneva			All subjects
		$3x10^6$ pfu (n=10)	$2x10^7$ pfu (n=10)	$3x10^5$ pfu (n=20)	$3x10^6$ pfu (n=19)	$3x10^6$ pfu (n=20)	$2x10^7$ pfu (n=20)	$1x10^7$ pfu (n=35)	$5x10^7$ pfu (n=16)	Placebo (=8)	(n=158)
Hemoglobin. g/L	Screening	148	150	135	138	143	146	143	144	142	**143**
	Day 1	141	147	131	135	-	-	140	141	139	**138**
	Day 2/3**	146	153	134	135	-	-	140	140	140	**140**
	Day 7	145	148	134	136	145	145	140	142	137	**141**
Platelets. G/L	Screening	271	256	203	211	246	255	246	235	226	**239**
	Day 1	230	224	193	188	-	-	228	215	232	**214**
	Day 2/3**	225	224	192	190	-	-	218	216	235	**211**
	Day 7	242	231	195	183	230	231	234	235	227	**223**
Leucocyte. G/L	Screening	7.9	6.6	6.3	6.6	5.6	5.0	5.9	6.6	5.7	**6.3**
	Day 1	5.7	6.3	5.7	5.8	-	-	5.8	5.9	5.6	**5.8**
	Day 2/3**	4.8	5.2	5.2	5.3	-	-	4.4	4.9	5.6	**4.9**
	Day 7	5.4	5.3	5.6	5.2	4.5	3.8	5.4	6.1	5.4	**5.2**
Absolute Lymphocyte count. G/L	Screening	1.8	1.9	2.3	2.3	2.5	2.2	2.0	2.0	2.0	**2.1**
	Day 1	0.8	0.7	1.8	1.2	-	-	0.9	0.9	2.0	**1.2**
	Day 2/3**	1.9	1.9	1.9	1.8	-	-	1.8	1.9	1.9	**1.9**
	Day 7	1.8	1.6	2.1	1.9	2.1	1.9	1.9	2.2	1.9	**1.9**
Absolute Neutrophil count. G/L	Screening	4.9	4.3	2.7	2.6	2.8	2.4	3.3	3.8	3.1	**3.1**
	Day 1	4.1	4.9	2.5	2.3	-	-	4.3	4.3	3.0	**3.6**
	Day 2/3**	2.0	2.4	1.9	1.8	-	-	2.0	2.2	3.1	**2.1**
	Day 7	2.9	2.9	1.9	1.7	2.0	1.6	2.9	3.2	2.9	**2.5**
Absolute Monocyte count. G/L	Screening	0.5	0.5	0.5	0.5	0.2	0.2	0.5	0.6	0.5	**0.4**
	Day 1	0.5	0.5	0.7	0.7	-	-	0.5	0.7	0.5	**0.6**
	Day 2/3**	0.6	0.6	0.6	0.7	-	-	0.5	0.7	0.5	**0.6**
	Day 7	0.4	0.4	0.5	0.4	0.2	0.2	0.5	0.5	0.4	**0.4**

Values are expressed as mean unless otherwise specified.
****Lambaréné: Day 2. Geneva and Hamburg: Day 3.**

Detailed Viremia Values

Table S5: Detailed viremia data within the first days after vaccination.

Day after injection	Hamburg				Lambaréné				Kilifi				Geneva					
	$3x10^6$ pfu n=10		$2x10^7$ pfu n=10		$3x10^5$ pfu n=20		$3x10^6$ pfu n=19		$3x10^6$ pfu n=20		$2x10^7$ pfu n=20		$1x10^7$ pfu n=35		$5x10^7$ pfu n=16		Placebo n=8	
	Number positive (%)	Copies/ml (IQR)	Number positive (%)	Copies/ml (IQR)	Number positive (%)	Copies/ml (IQR)	Number positive (%)	Copies/ml (IQR)	Number positive (%)	Copies/ml (IQR)	Number positive (%)	Copies/ml (IQR)	Number positive (%)	Copies/ml (IQR)	Number positive (%)	Copies/ml (IQR)	Number positive (%)	Copies/ml (IQR)
0	0%	ND	0%	ND	0%	ND	0%	ND	0%	ND	5%	ND	0%	ND	0%	ND	0%	ND
1	100%	50 (50)	100%	50 (50)	11%* (2/18)	ND	90%	224 (98-606)	75%	65 (44-417)	100%* (19/19)	811 (523-1185)	86%	273 (65-911)	94%	377 (106-866)	0%	ND
2	100%	50 (50-265)	90%	313 (50-649)	26%* (5/19)	ND (ND-40)	95%* (17/18)	750 (256-1657)	-	-	-	-	90%* (9/10)	138 (65-733)	100%* (8/8)	596 (189-2285)	0%* (0/5)	ND
3	90%	50 (50)	100%	50 (50-339)	-	-	-	-	95%	579 (299-912)	100%	1135 (172-1373)	76%* (19/25)	293 (65-707)	50%* (4/8)	40 (ND-149)	0%* (0/3)	ND
4	30%	ND (ND-38)	70%	50 (13-50)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	10%	ND	0%* (0/9)	ND	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	0%	ND	0%	ND	8%* (1/12)	ND	22%* (4/18)	ND	10%	ND	15%	ND	3%	ND	0%	ND	0%* (0/6)	ND
>7	0%	ND	0%	ND	-	-	-	-	-	-	-	-	1**	ND	-	-	-	-

Copies/ml is represented as median of one dose cohort.
ND = Not detectable. - = Not analyzed.
* Incomplete data set . Given in brackets is the number of subjects positive versus total subjects analyzed.
** Samples were only assessed > day 7 if viremia was still detected on day 7 (n=1) or in subjects with arthritis and/or skin lesions (n=6).

Description of Arthritis Cases in Geneva

Table S6: Arthritis case descriptions in Geneva.

Case n°	Age (years)	Gender	Treatment received	Day of onset post-injection	Joint(s) affected (total number)	Imaging: type, findings (study day done)	Pain		Morning stiffness (> 30 minutes)	DAS 44** [15]	Skin lesion(s)
							Total duration (days)	Maximal grade* (days)			
1	40	M	10^7 pfu	11	R MCP3, R PIP3 (2)	Not done	6º	1 (6)	Present	1.96	None
2	40	F	10^7 pfu	9	L MCP2, L PIP2, R wrist (3)	US: L MCP2, L PIP2 synovitis (15)	9	1 (9)	None	2.24	None
3	34	M	10^7 pfu	14	R ankle, LMCP2 (2)	US: R talo-crural/subtalar and L MCP2 arthritis (14)	4	1 (4)	None	1.79	None
4	45	F	10^7 pfu	9	R MCP1, R MCP5, R MTP1, L knee (4)	US: R MTP1 arthritis (11)	167§	3 (2)	Present	2.22	Generalized papular rash, one vesicle
5	43	M	10^7 pfu	9	L5-S1 (1)	MRI: degenerative changes L5-S1 (17)	79§	3 (1)	Present	ND	None
6	50	F	10^7 pfu	6‡	R MCP3, R MCP4, R PIP3, R elbow (4)	US: resolving inflammation (9)	2‡	1 (2)	Present	1.86	Papular rash on hands, feet and knees; vesicles
7	24	F	10^7 pfu	14	L Coxo-femoral, L sacro-iliac (2)	MRI: interepinous bursitis L4-L5 (30)	87§	3 (8)	Present	1.78	None

8	41	M	$5x10^7$ pfu	18	Knees (2)	US: arthritis with effusion in L knee (20)	8†	2 (3)	Present	1.41	Papular rash hands and feet; vesicles
9	51	M	10^7 pfu	10	R MCP5, knees (3)	US: arthritis R knee, discrete effusion L knee (rVSV+) (15)	7	3 (1)	Present	1.74	None
10	24	M	$5x10^7$ pfu	14	R MTP1, wrists (3)	US: L extensor tenosynovitis (15)	3	3 (1)	None	1.52	None
11	35	F	$5x10^7$ pfu	12	L coxo-femoral (1)	US: non-specific findings (31)	109§	3(3)	None	ND	None

The median interval between injection and symptom onset was 11 days, the median duration of pain 8 days (IQR 4-87).

MCP: metacarpophalangeal joint; MTP: metatarsophalangeal joint; PIP: proximal interphalangeal joint; US: ultrasound; MRI: magnetic resonance imaging.

*Pain intensity: grade 1 = no interference with activity; 2= some interference with activity; 3= significant, prevents daily activity; 4= medical consultation and/or hospitalization required.

**The DAS44 (Disease Activity Score 44) is based on the number of joints involved/swollen and erythrocyte sedimentation rate.

º This subject experienced a possible recurrence 4 months after initial resolution, including mild to moderate arthralgia in the previously unaffected right MCP4 and finger PIP4 joints.

§ After the first two weeks, pain was mild and mainly present upon movement.

‡This subject experienced a suspected relapse on day 82 with clinical arthritis in three right-sided finger joints and one tender vesicle identical to those that had appeared 2.5 months before. Symptoms were mild and self-limited, with full resolution after 10 days.

† Initial pain resolved after 10 days, but a sequela of occasional (≤ 1x/week), mild, residual pain in both knees was later reported, persisting for another 134 days.

Description of Arthritis Cases in Kilifi and Hamburg

Clinical arthritis case 1: Kilifi -Subject 25 (Arthritis diagnosed clinically).

This 43 year old Caucasian female participant was vaccinated with $3x10^6$ pfu on 19 January 2015. She has a history of ankylosing spondylitis but has not had any active flare in over 20 years. She did not experience any initial reactogenicity. On day 9, at dinner on standing she experienced a sharp pain in the right knee described as a "dead leg" but did not contact study personnel as she did not link it to the study intervention. She was however able to walk but had to take some ibuprofen that night for the pain. The following morning (day 10) she woke up with a painful right knee, which worsened over the day and again required a nocturnal dose of ibuprofen. On day 11, she contacted the study personnel; consultation revealed grade 2 arthralgia and arthritis of the right knee joint that was associated with a slight limp. There was no history of trauma, febrile illness or other injuries. There was no associated rash. Examination of the knee showed swelling around the knee with some limitation on flexion of the joint. Patellar tap was negative and did not suggest significant joint effusion. She was prescribed ibuprofen 400mg as needed. On day 12, she reported resolution of the swelling, pain and the limp. On day 14, she was without complaints overall and remained afebrile. There was still some residual stiffness in the morning or following long periods of sitting. No laboratory examination was performed at the time of joint symptoms. Imaging by ultrasound was offered but declined by the subject. In summary, history and clinical presentation were compatible with a reactive arthritis following immunization. Causality was considered probable.

Clinical arthritis case 2: Hamburg -Subject 102 (former gonarthritis of right knee diagnosed clinically, subsequently diagnosed as retropatellar chondropathy/gonarthrosis).

This 26-year-old male student was vaccinated with $3x10^6$ pfu rVSV-ZEBOV on November 20th 2014. He experienced transient reactogenicity on days 0-3 with myalgia, fever (38.3°C) and mild pain at injection

site. On day 8 after vaccination he awoke at night with right knee pain and over the ensuing days experienced grade 3 arthralgia of the right knee with severe pain and subjective slight swelling that led to ibuprofen intake and physical rest on days 8 – 22. He presented to an orthopedic outpatient department on day 11 post-injection. No significant joint swelling was observed, no significant effusion was elicited on exam, therefore no imaging or arthrocentesis was performed. He was medicated with ibuprofen. He had reported these complaints to the study team at onset but had initially assumed that the joint pain was related to trauma (twisted knee?). However, the subject contacted the study team again for further evaluation only on day 21 after the email alert provided by the study team outlining the observed reactive arthritis reported from Geneva. No trauma or physical exercise was reported. No general malaise, no other signs of sickness were present when arthritis started. No rashes or other skin lesions were observed. He reported a similar short previous episode of unexplained right-sided knee pain when he was 18 years old (no clear etiology was established at the time). Apart from this episode he had no history of any joint or bone disorder or trauma. The subject had no family history of rheumatic disorders or documented reactive arthritis, however his father was said to suffer from „degenerative knee problems“, possibly starting in his young adulthood. At an invited unscheduled visit on day 22 he had no pain and the right knee was without abnormalities on examination. A rheumatologist was additionally consulted. Ultrasound imaging of the knee revealed minimally increased joint fluid without drainable effusion was without complaints with full resolution of symptoms. History and clinical presentation were initially considered as compatible with a reactive arthritis following immunization, and causality as probable.

Update at time of writing of this manuscript: After recurrence of mild knee discomfort the study subject reconsulted an orthopedist around 50 days p.i. (January 2015). An MRI evaluation was performed at this time identified underlying structural changes indicative of retropatellar chondropathy and gonarthrosis. The outside orthopedic specialist consulted by the study subject suggested that the knee symptoms were likely degenerative and unrelated to vaccination, however a possible element of reactive arthritis at the time of initial presentation cannot fully be ruled out.

Study flow diagram.

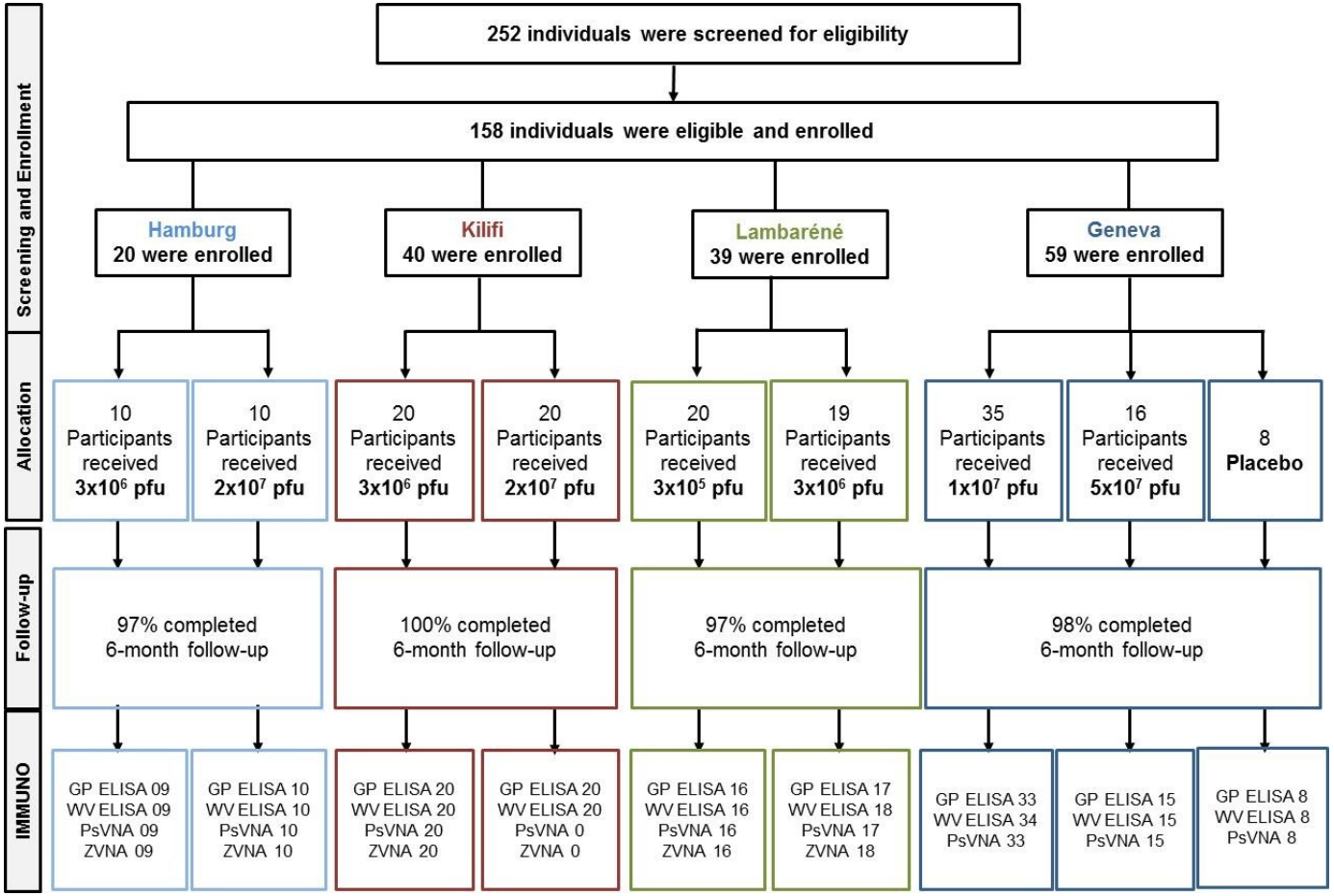


Figure S4: Clinical Trial Profile. Flow diagram of the progress of harmonized open-label and double-blind placebo-controlled dose escalation Phase I trials in four sites. Flow chart represents study designs indicating the numbers of screened and enrolled individuals, as well as the completion of follow-up visits. 51/252 (20%) subjects were excluded due to inegibility (Hamburg 9, Lambaréné 18, Kilifi 14, Geneva 10) and 43 were eligible but not dosed. Eight participants were not included in month-6 ITT analyses (missing samples [2], pregnancy [2], HIV [1], varia [3]).

Immunogenicity

Antibody responses measured by ZEBOV-GP ELISA

Table S7 - End-point geometric mean titers (GMT), seropositivity rates and proportion of seroresponses to rVSV-ZEBOV at various time intervals, measured by ZEBOV-GP ELISA.*

		N	GMT (95%CI)	p value GMT days 0 vs 28	p value GMT days 0 vs 180	p value GMT days 28 vs 180	Geometric mean ratio of D180/D28 titers	Seropositivity (titer ≥ 50), n (%)	p value seropositivity vs day 0
Geneva									
Placebo	Day 0	8	25 (-)					0 (0.0)	
	Day 28	8	25 (-)	NA				0 (0.0)	NA
	Day 180	8	25 (-)		NA	NA	1.00 (-)	0 (0.0)	NA
$1x10^7$	Day 0	34	33.9 (26.6 to 43.4)					8 (23.5)	
	Day 28	34	1064.2 (757.6 to 1495.1)	<0.0001				34 (100.0)	<0.0001
	Day 180	33	1634.0 (1198.5 to 2227.7)		<0.0001	0.0044	1.59 (1.21 to 2.09)	33 (100.0)	<0.0001
$5x10^7$	Day 0	13	36.3 (26.1 to 50.5)					5 (38.5)	
	Day 28	13	1780.1 (1048.3 to 3022.5)	0.0016				13 (100.0)	0.0078
	Day 180	15	1837.9 (1179.3 to 2864.3)		0.0016	0.892	1.05 (0.57 to 1.97)	15 (100.0)	0.0078
Lambarene									
$3x10^5$	Day 0	20	42.0 (31.9 to 55.4)	<0.0001				11 (55.0)	0.0039
	Day 28	20	1055.6 (520.7 to 2139.9)					20 (100.0)	
	Day 180	16	712.9 (365.7 to 1389.7)		0.0007	0.6137	0.89 (0.56 to 1.43)	15 (93.8)	0.0313
$3x10^6$	Day 0	19	38.7 (24.0 to 62.5)	0.0002				3 (18.8)	<0.0001
	Day 28	19	2570.9 (1512.9 to 4369.1)					19 (100.0)	
	Day 180	17	1359.2 (899.9 to 2052.9)		0.0004	0.0428	0.59 (0.35 to 0.98)	17 (100.0)	0.0001

NA denotes not applicable.
† Seropositivity was defined as an end-point titer of 50 or more

Table S7 (cont) - End-point geometric mean titers (GMT), seropositivity rates and proportion of seroresponses to rVSV-ZEBOV at various time intervals, measured by ZEBOV-GP ELISA.*

		N	GMT (95%CI)	p value GMT days 0 vs 28	p value GMT days 0 vs 180	p value GMT days 28 vs 180	Geometric mean ratio of D180/D28 titers	Seropositivity (titer ≥ 50), n (%)	p value seropositivity vs day0
Kilifi									
$3x10^6$	Day 0	20	33.0 (23.0 to 47.3)					3 (15.0)	
	Day 28	20	1492.9 (995.5 to 2238.6)	<0.0001				20 (100.0)	<0.0001
	Day 180	20	1392.6 (968.4 to 2003.4)		<0.0001	0.7909	0.93 (0.63 to 1.38)	20 (100.0)	<0.0001
$2x10^7$	Day 0	0	NA						
	Day 28	0	NA	NA				NA	NA
	Day 180	20	1600 (1137.2 to 2251.1)		NA	NA	NA	20 (100.0)	NA
Hamburg									
$3x10^6$	Day 0	10	25 (-)	0.0055				0 (0.0)	0.0020
	Day 28	10	1392.9 (893.7 to 2170.8)					10 (100.0)	
	Day 180	9	903.9 (506.7 to 1612.2)		0.0085	0.3973	0.71 (0.40 to 1.26)	9 (100.0)	0.0039
$2x10^7$	Day 0	10	30.8 (23.0 to 41.1)	0.0056				2 (20.0)	0.0078
	Day 28	10	1969.8 (1249.6 to 3105.2)					10 (100.0)	
	Day 180	10	1600.0 (974.3 to 1612.2)		0.0058	0.3741	0.81 (0.54 to 1.22)	10 (100.0)	0.0078

NA denotes not applicable.

† Seropositivity was defined as an end-point titer of 50 or more.

Table S8: End-point geometric mean titers (GMT), seropositivity rates and proportion of seroresponders to rVSV-ZEBOV measured by ZEBOV-GP ELISA (per-protocol population, Geneva).

		N	GMT (95%CI)	Geometric mean ratio of D180/D28 titers	p value GMT days 0 vs 28	p value GMT days 0 vs 180	p value GMT days 28 vs 180	Seropositivity °, n (%)	Seroresponse, n (%)	p value Seropositivity days 0 vs 28	p value Seropositivity days 0 vs 180
Geneva											
Placebo	Day 0	8	25 (-)					0 (0.0)			
	Day 28	8	25 (-)		NA			0 (0.0)	0 (0.0)	NA	
	Day 180	8	25 (-)	1.00 (-)		NA	NA	0 (0.0)	0 (0.0)		NA
$1x10^7$	Day 0	27	30.7 (25.4 to 37.1)					5 (18.5)			
	Day 28	27	1034.1 (713.7 to 1498.4)		<0.0001			27 (100.0)	27 (100.0)	<0.0001	
	Day 180	26	1687.6 (1195.6 to 2382.2)	1.70 (1.22 to 2.37)		<0.0001	0.0413	26 (100.0)	26 (100.0)		<0.0001
$5x10^7$	Day 0	11	36.5 (24.9 to 53.5)					4 (36.4)			
	Day 28	11	1600 (896.5 to 2855.6)		0.0038			11 8100.0)	11 (100.0)	0.0156	
	Day 180	13	1600 (1065.0 to 2403.7)	1.00 (0.56 to 1.78)		0.0037	0.6326	13 (100.0)	11 (100.0)		0.0156

Results are expressed as endpoint titers with 95% confidence intervals. Seropositivity is defined by an end-point titer ≥ 50.
Seroresponse is defined by a ≥ 4-fold rise in endpoint titers.

Antibody responses measured by whole-virion ELISA

Tables S9A & B: Geometric mean concentration (GMC), seropositivity rates and proportion of seroresponders to rVSV-ZEBOV measured by whole-virion ELISA.

A. Per-protocol population (Geneva only):

		N	GMT (95%CI)	Geometric mean ratio of D180/D28 titers	p value GMT days 0 vs 28	p value GMT days 0 vs 180	p value GMT days 28 vs 180	S+°, n (%)	SR, n (%)	p value Seropositivity days 0 vs 28	p value Seropositivity days 0 vs 180
Geneva											
Placebo	Day 0	8	564.2 (445.2 to 715.0)		1			1 (12.5)		1.0000	
	Day 14	8	500 (-)					0 (0.0)	0 (0.0)		
	Day 28	8	500 (-)					0 (0.0)	0 (0.0)		
	Day 180		500 (-)	1.00 (-)		1	NA	0 (0.0)	0 (0.0)		1
$1x10^7$	Day 0	28	524.4 (477.7 to 575.7)		0.0025			1 (3.7)		0.0010	
	Day 14	28	593.6 (490.5 to 718.4)					3 (10.7)	2 (7.1)		
	Day 28	27	1023.2 (728.0 to 1438.1)					12 (44.4)	6 (22.2)		
	Day 180	27	769.3 (584.5 to 1012.3)	0.77 (0.58 to 1.01)		0.0209	0.1261	8 (29.6)	4 (14.8)		0.0156
$5x10^7$	Day 0	14	700.6 (490.8 to 1000.0)		0.036			3 (21.4)		0.1250	
	Day 14	14	842.4 (566.7 to 1252.3)					5 (35.7)	0 (0.0)		
	Day 28	12	1125.5 (661.7 to 1914.4)					6 (50.0)	1 (8.3)		
	Day 180	13	691.3 (483.7 to 988.0)	0.57 (0.36 to 0.90)		0.7893	0.036	3 (23.1)	0 (0.0)		1

Results are expressed in arbitrary ELISA units (AEU)/ml with 95% confidence intervals. Seropositivity is defined by a GMT > 500 AEU/ml. Seroresponse is defined by a ≥ 4-fold rise in endpoint titers.

B. Intention-to-treat population:

		N	GMT (95%CI)	Geometric mean ratio of D180/D28 titers	p value GMT days 0 vs 28	p value GMT days 0 vs 180	p value GMT days 28 vs 180	Seropositivity n (%)	Seroresponse n (%)	p value Seropositivity days 0 vs 28	p value Seropositivity days 0 vs 180
Geneva											
Placebo	Day 0	8	564.2 (445.2 to 715.0)					1 (12.5)			
	Day 28	8	500 (-)		1			0 (0.0)	0 (0.0)	1.0000	
	Day 180		500 (-)	1.00 (-)		1	NA	0 (0.0)	0 (0.0)		1
$1x10^7$	Day 0	35	555.5 (478.4 to 645.1)					2 (5.7)			
	Day 28	34	983.0 (721.3 to 1339.7)		0.0011			14 (41.2)	6 (17.6)	0.0005	
	Day 180	34	836.1 (634.4 to 1102.0)	0.87 (0.66 to 1.14)		0.0067	0.2446	11 (32.4)	5 (14.7)		0.0039
$5x10^7$	Day 0	16	671.6 (490.2 to 920.3)					3 (18.8)			
	Day 28	14	1201.3 (707.7 to 2039.4)		0.0225			7 (50.0)	2 (14.3)	0.0625	
	Day 180	15	695.2 (507.0 to 953.4)	0.54 (0.32 to 0.91)		0.3613	0.03	4 (26.7)	0 (0.0)		0.5
Lambarene											
$3x10^5$	Day 0	19	574.9 (437.3 to 755.7)		0.0033			1 (5.3)			
	Day 28	20	1887.1 (1154.2 to 3085.3)					13 (65.0)	10 (52.6)	0.0010	
	Day 180	16	1193.6 (772.4 to 1884.5)	0.80 (0.50 to 1.27)		0.0432	0.3505	9 (56.3)	5 (33.3)		0.0156
$3x10^6$	Day 0	19	641.1 (486.5 to 844.9)		0.0059			3 (15.8)			
	Day 28	19	1426.8 (825.3 to 2466.6)					10 (52.6)	6 (31.6)	0.0156	
	Day 180	18	1154.3 (742.3 to 1795.0)	0.79 (0.60 to 1.05)		0.00915	0.1029	9 (50.0)	3 (16.7)		0.03125
Kilifi											
$3x10^6$	Day 0	20	547.2 (484.3 to 618.3)					2 (10.0)			
	Day 28	20	1123.9 (705.0 to 1791.7)		0.0143			8 (40.0)	6 (30.0)	0.0313	
	Day 180	20	1278.7 (906.6 to 1803.6)	1.14 (0.76 to 1.69)		0.0017	0.5294	13 (65.0)	8 (40.0)		0.001
$2x10^7$	Day 0	18	578.1 (435.0 to 768.3)					1 (5.6)			
	Day 28	20	1131.4 (731.5 to 1749.7)		0.0129			10 (50.0)	5 (27.8)	0.0078	
	Day 180	20	883.3 (639.2 to 1220.6)	0.78 (0.48 to 1.26)		0.0519	0.5935	8 (40.0)	3 (16.7)		0.0313
Hamburg											
$3x10^6$	Day 0	10	500 (-)					0 (0.0)			
	Day 28	10	500 (-)		NA			0 (0.0)	0 (0.0)	NA	
	Day 180	9	561.7 (447.2 to 705.4)	1.12 (0.89 to 1.41)		1	1	1 (11.1)	0 (0.0)		1
$2x10^7$	Day 0	10	500 (-)					0 (0.0)			
	Day 28	10	920.7 (541.2 to 1566.4)		0.1003			4 (40.0)	3 (30.0)	0.1250	
	Day 180	10	870.0 (581.3 to 1302.2)	0.94 (0.57 to 1.58)		0.0591	0.7874	5 (50.0)	1 (10.0)		0.0625

Results are expressed in arbitrary ELISA units (AEU)/ml with 95% confidence intervals. Seropositivity is defined by a GMT > 500 AEU/ml. Seroresponse is defined by a ≥ 4-fold rise in endpoint titers.

Antibody responses measured by pseudovirus neutralisation

Tables S10A & B: Pseudovirus neutralization 50 geometric mean titer (GMT), seropositivity rates and proportion of seroresponders to rVSV-ZEBOV.

A. Per-protocol population (Geneva only):

		N	GMT (95%CI)	Geometric mean ratio of D180/D28 titers	p value GMT days 0 vs 28	p value GMT days 0 vs 180	p value GMT days 28 vs 180	Seropositivity °, n (%)	Seroresponse, n (%)	p value Seropositivity days 0 vs 28	p value Seropositivity days 0 vs 180
Geneva											
Placebo	Day 0	8	10 (-)		NA			0 (0.0)		NA	
	Day 28	8	10 (-)					0 (0.0)	0 (0.0)		
	Day 180	8	10 (-)	1.00 (-)		NA	NA	0 (0.0)	0 (0.0)		NA
$1x10^7$	Day 0	34	10 (-)		<0.0001			0 (0.0)		<0.0001	
	Day 28	34	99.1 (61.9 to 158.8)					30 (88.2)	24 (70.6)		
	Day 180	33	18.4 (12.8 to 26.4)	0.18 (0.12 to 0.27)		0.0092	<0.0001	9 (27.3)	9 (27.3)		0.0039
$5x10^7$	Day 0	13	10 (-)		0.0002			0 (0.0)		0.0002	
	Day 28	13	272.7 (156.6 to 475.0)					13 (100.0)	13 (100.0)		
	Day 180	15	17.9 (10.6 to 30.1)	0.06 (0.03 to 0.12)		0.1814	0.0002	4 (26.7)	3 (23.1)		0.2500

Results are expressed as PsVNA50 neutralization titers with 95% confidence intervals. Seropositivity is defined by a GMT > 20 of 2 replicates. Seroresponse is defined by a ≥ 4-fold rise in endpoint titers.

B. Intention-to-treat population:

		N	GMT (95%CI)	Geometric mean ratio of D180/D28 titers	p value GMT days 0 vs 28	p value GMT days 0 vs 180	p value GMT days 28 vs 180	Seropositivity n (%)	Seroresponse n (%)	p value Seropositivity days 0 vs 28	p value Seropositivity days 0 vs 180
Geneva											
Placebo	Day 0	8	10 (-)		NA			0 (0.0)		NA	
	Day 28	8	10 (-)					0 (0.0)	0 (0.0)		
	Day 180	8	10 (-)	1.00 (-)		NA	NA	0 (0.0)	0 (0.0)		NA
$1x10^7$	Day 0	34	10 (-)		<0.0001			0 (0.0)		<0.0001	
	Day 28	34	99.1 (61.9 to 158.8)					30 (88.2)	24 (70.6)		
	Day 180	33	18.4 (12.8 to 26.4)	0.18 (0.12 to 0.27)		0.0092	<0.0001	9 (27.3)	9 (27.3)		0.0039
$5x10^7$	Day 0	13	10 (-)		0.0002			0 (0.0)		0.0002	
	Day 28	13	272.7 (156.6 to 475.0)					13 (100.0)	13 (100.0)		
	Day 180	15	17.9 (10.6 to 30.1)	0.06 (0.03 to 0.12)		0.1814	0.0002	4 (26.7)	3 (23.1)		0.2500
Lambarene											
$3x10^5$	Day 0	20	10 (-)		0.0039			0 (0.0)		0.0010	
	Day 28	20	49.7 (22.9 to 108.0)					11 (55.0)	10 (50.0)		
	Day 180	16	11.6 (8.6 to 15.7)	0.24 (0.11 to 0.52)		1	0.0092	1 (6.3)	1 (6.3)		1
$3x10^6$	Day 0	19	10 (-)		0.0003			0 (0.0)		<0.0001	
	Day 28	19	85.5 (45.6 to 160.3)					2 (10.5)	13 (68.4)		
	Day 180	17	10.7 (9.3 to 12.4)	0.14 (0.07 to 0.28)		1	0.0007	1 (5.9)	0 (0.0)		1
Kilifi											
$3x10^6$	Day 0	20	10 (-)					0 (0.0)			
	Day 28	20	67.3 (38.9 to 116.7)		0.0005			16 (80.0)	13 (65.0)	<0.0001	
	Day 180	20	18.0 (12.1 to 26.7)	0.27 (0.17 to 0.42)		0.0225	0.0006	7 (35.0)	5 (25.0)		0.0156
$2x10^7$	Day 0	0	NA								
	Day 28	0	NA								
	Day 180	0	NA								
Hamburg											
$3x10^6$	Day 0	10	10 (-)		0.0020			0 (0.0)		0.0020	
	Day 28	10	96.2 (62.9 to 147.3)					10 (100.0)	10 (100.0)		
	Day 180	9	16.5 (8.3 to 32.8)	0.17 (0.07 to 0.44)		0.3711	0.0117	2 (22.2)	2 (22.2)		0.1250
$2x10^7$	Day 0	10	10 (-)		0.0020			0 (0.0)		0.0020	
	Day 28	10	167.7 (120.6 to 233.1)					10 (100.0)	10 (100.0)		
	Day 180	10	18.6 (11.0 to 31.4)	0.11 (0.08 to 0.16)		0.1003	0.0020	4 (40.0)	2 (20.0)		0.5000

Results are expressed as PsVNA50 neutralization titers with 95% confidence intervals. Seropositivity is defined by a GMT > 20 of 2 replicates. Seroresponse is defined by a ≥ 4-fold rise in endpoint titers.

Antibody responses measured by ZEBOV virus neutralization

Table S11: Geometric mean titers (GMT), seropositivity rates and proportion of seroresponders to rVSV-ZEBOV measured by ZEBOV virus neutralization (intention-to-treat population):

			GMT (95%CI)	Geometric mean ratio of D180/D28 titers	p value GMT days 0 vs 28	p value GMT days 0 vs 180	p value GMT days 28 vs 180	Seropositivity n (%)	Seroresponse n (%)	p value Seropositivity days 0 vs 28	p value Seropositivity days 0 vs 180
Lambarene											
$3x10^5$	Day 0	19	6.5 (4.9 to 8.7)					7 (36.8)			
	Day 28	20	18.1 (10.5 to 30.9)		0.0045			14 (70.0)	8 (42.1)	0.0313	
	Day 180	16	8.8 (6.5 to 11.8)	0.44 (0.21 to 0.90)		0.5047	0.0729	6 (37.5)	2 (13.3)		1
$3x10^6$	Day 0	19	4.3 (3.9 to 4.7)					0 (0.0)			
	Day 28	19	15.4 (10.1 to 23.5)		0.0006			15 (78.9)	10 (52.6)	<0.0001	
	Day 180	18	11.2 (9.1 to 13.8)	0.67 (0.43 to 1.05)		0.0002	0.0428	14 (77.8)	5 (27.8)		0.0001
Kilifi											
$3x10^6$	Day 0	20	4.5 (4.0 to 5.2)					2 (10.0)			
	Day 28	20	9.2 (7.1 to 11.9)		0.0007			13 (65.0)	2 (10.0)	0.0010	
	Day 180	20	9.0 (7.0 to 11.7)	0.99 (0.73 to 1.33)		0.0007	0.7936	10 (50.0)	4 (20.0)		0.0078
$2x10^7$	Day 0	20	5.1 (4.4 to 5.9)					1 (5.0)			
	Day 28	20	13.7 (9.6 to 19.4)		0.0003			10 (50.0)	7 (35.0)	0.0039	
	Day 180	0	NA								
Hamburg											
$3x10^6$	Day 0	10	6.1 (4.6 to 8.2)					2 (20.0)			
	Day 28	10	15.5 (9.7 to 24.7)		0.0128			9 (90.0)	3 (30.0)	0.0156	
	Day 180	9	13.4 (11.3 to 15.9)	0.86 (0.46 to 1.59)		0.0128	0.6741	9 (100.0)	3 (33.3)		0.0156
$2x10^7$	Day 0	10	4.4 (3.6 to 5.4)					1 (10.0)			
	Day 28	10	22.2 (15.7 to 31.4)		0.0059			10 (100.0)	6 (60.0)	0.0039	
	Day 180	10	11.3 (8.9 to 14.4)	0.51 (0.33 to 0.79)		0.0058	0.0273	6 (60.0)	3 (30.0)		0.0625

Results are expressed as neutralization titers with 95% confidence intervals. Seropositivity is defined by a GMT > 8. Seroresponse is defined by a ≥ 4-fold rise in endpoint titers.

Illustration of antibody responses measured by whole-virion ELISA

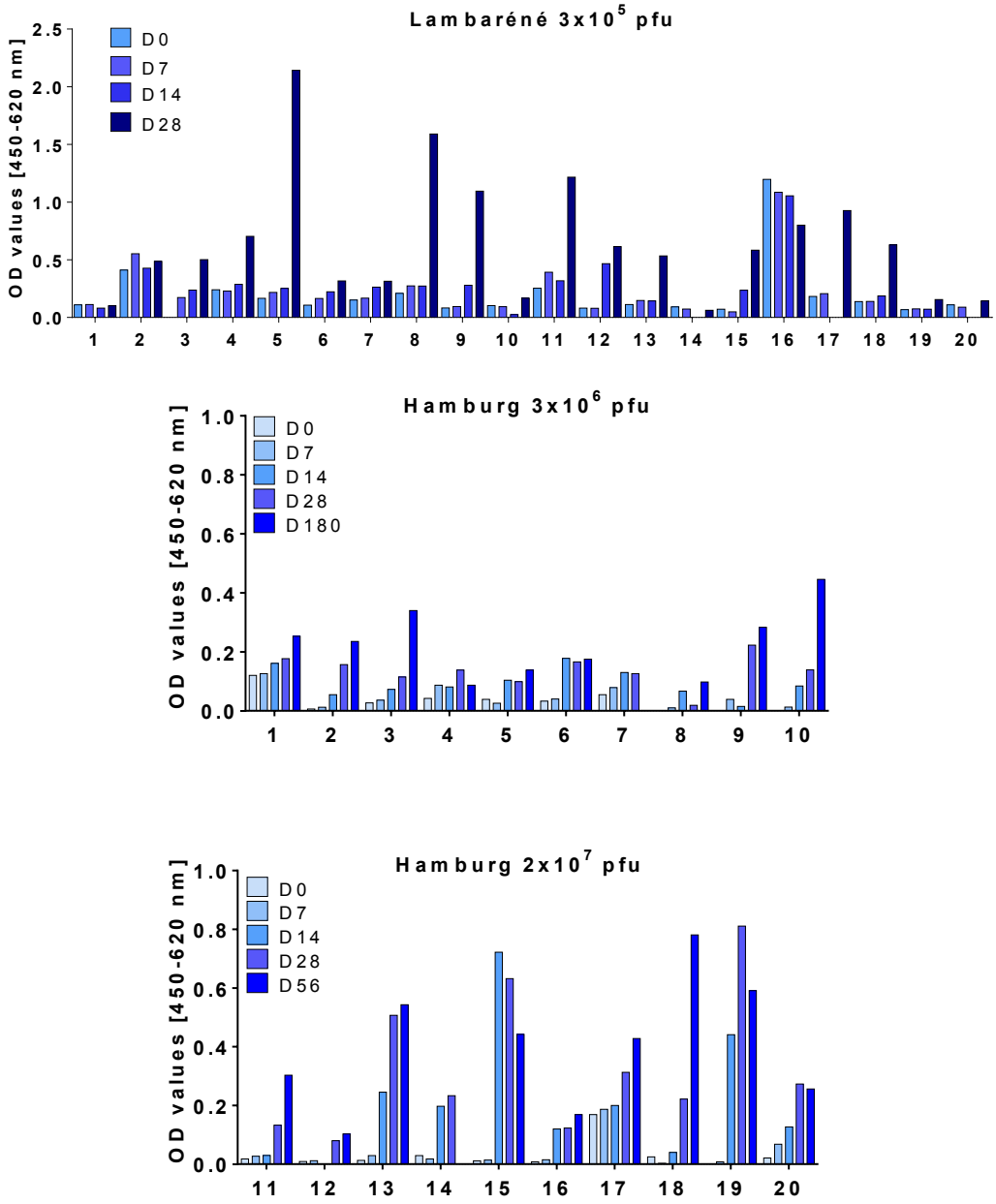


Figure S5: Whole Virion ELISA Assay - Increase of Optical Density Values in Three Study Cohorts.

Mean OD value determined by ELISA.

Serum samples of three dose cohorts (A, Lambaréné $3x10^5$, B, Hamburg $3x10^6$ and C, Hamburg $2x10^7$ pfu) were analyzed via whole-virion ELISA on day 0, 7, 14 and 28 and 180). The graph represents the optical density values [OD=450 – 620 nm]) of each individual participant (1 – 20). It demonstrates that subjects from Hamburg receiving $2x10^7$ PFU did respond to immunization by an increase in OD.

Antibody responses measured by Western Blot

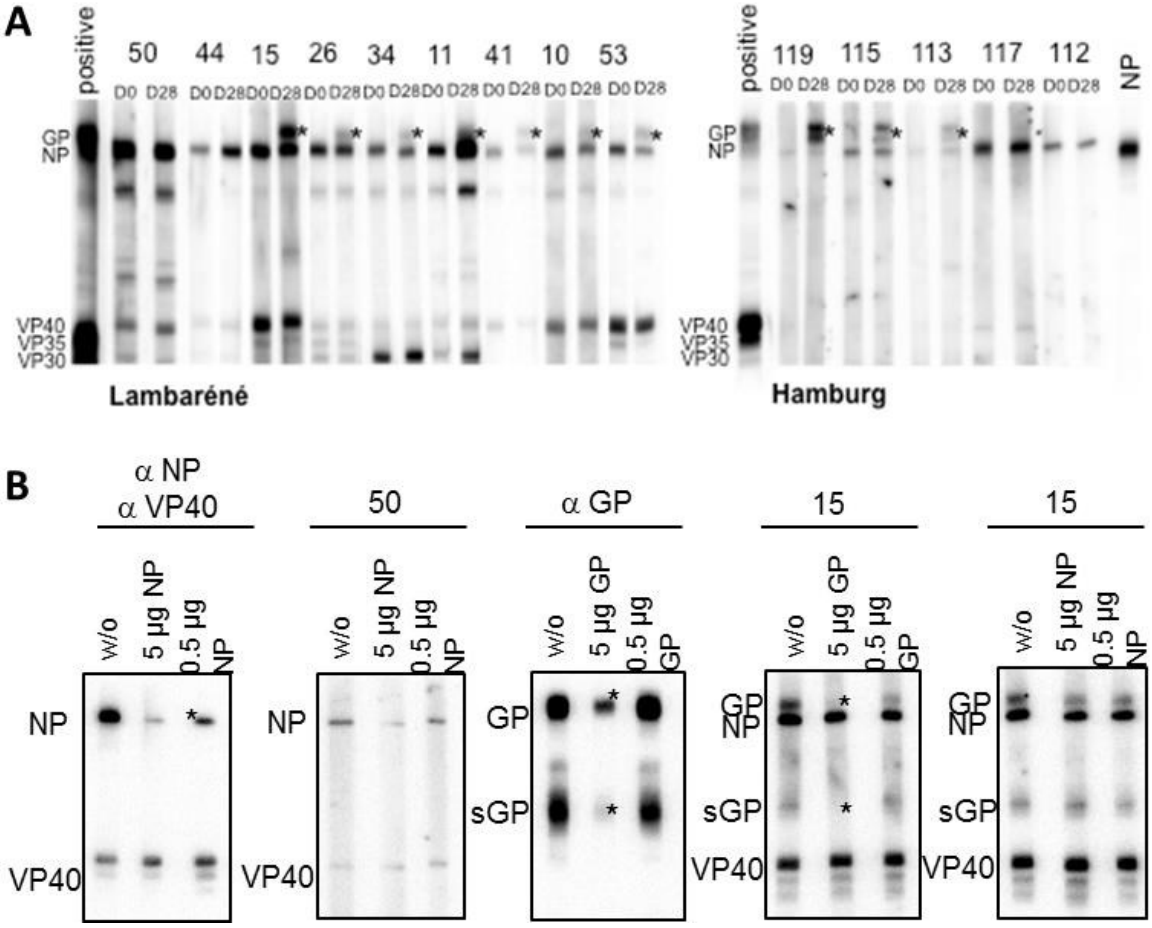


Figure S6: Western Blot analysis of sera from Hamburg and Lambaréné cohorts.

(A) Sera from volunteers day 0 and day 28 or day 14 post-vaccination were examined by Western blot analysis. Left panel: sera from the Lambaréné cohort ($3x10^5$ pfu). Right panel: Sera from the Hamburg cohort ($2x10^7$ pfu). Migration pattern of filoviral proteins are indicated at the left. Asterisks highlight a positive GP signal. (B) Specificity of Western blot signals. Sera of volunteers from Lambaréné (#15 and #50), control antibodies directed against EBOV-NP and EBOV-VP40 (anti-NP/anti-VP40), or control antibody directed against EBOV-GP (anti-GP) were pre-incubated for 1 hour with 5 or 0.5 µg of recombinant EBOV-NP (anti NP/anti VP40, #15 and #50) or recombinant EBOV-GP (anti-GP and #15). Sera were then subjected to Western blot analysis. Detection of bound antibodies was performed with POD-coupled secondary antibodies. Asterisks: diminished detection due to pre-incubation of antibodies with respective recombinant proteins.

Correlation analyses between antibody assays

Method: Spearman correlation coefficients (all centers, any dose, including placebo for Geneva).

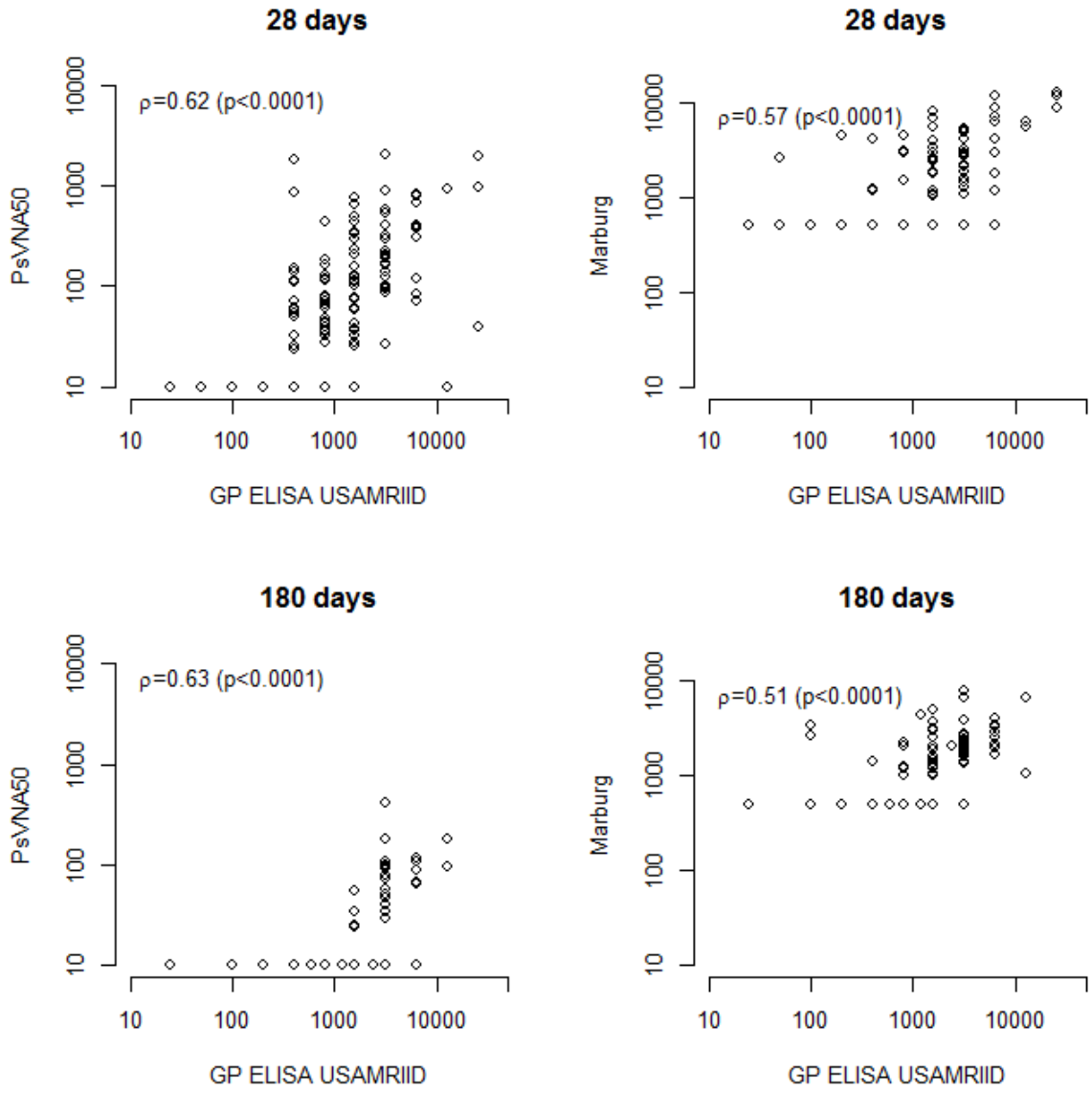


Figure S7: Correlation analyses between assays.

The strongest positive correlation was observed between results of the GP-based ELISA (GP ELISA UASMRIID) and the PsVNA50 assay. This correlation persisted at 6 months.

Association between doses and GMT/GMC

A. Day 28

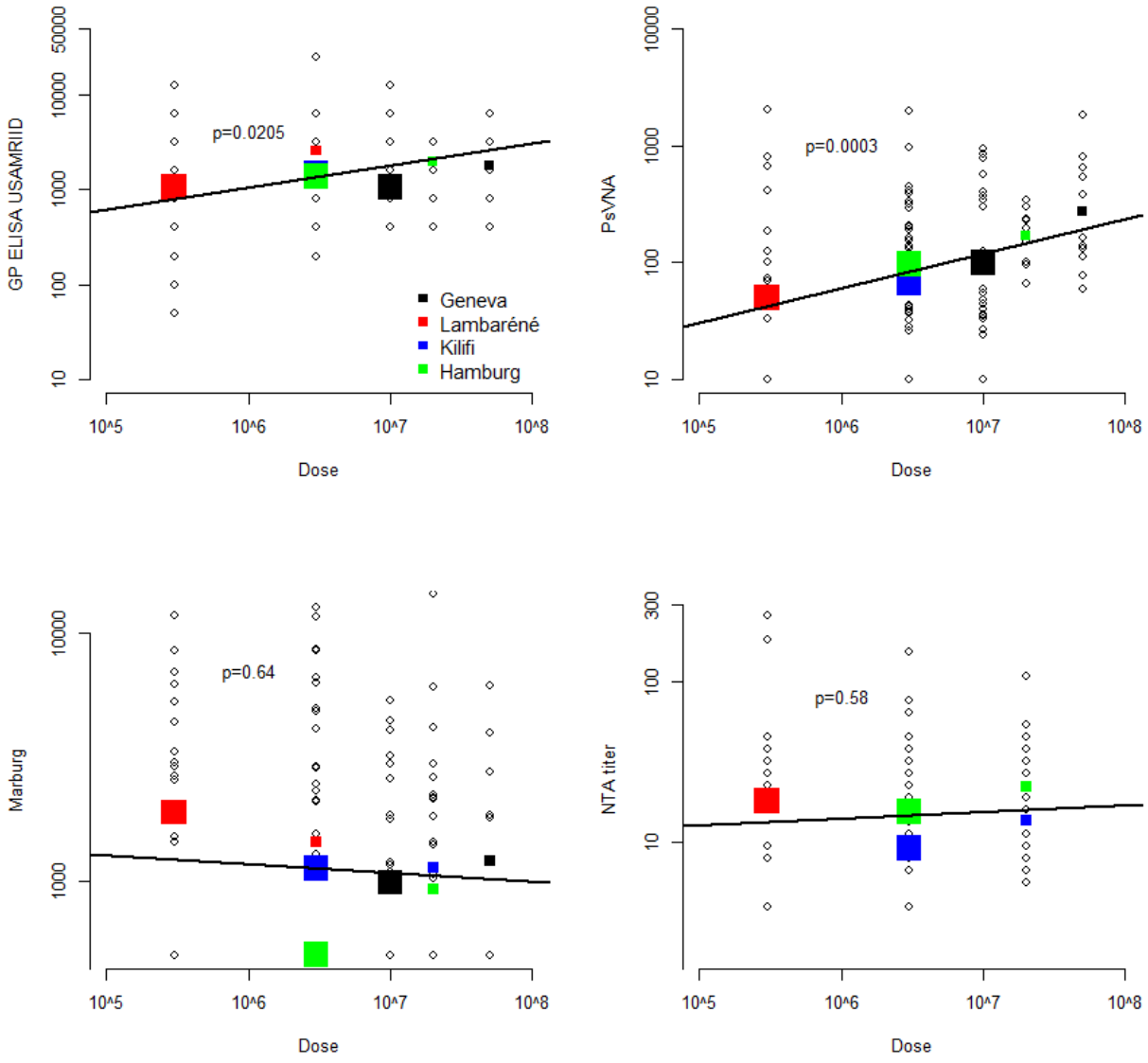


Figure S8A: Association between doses and day 28 GMT/GMC.

Method: linear regression with mixed effects on intercept (for potential center effect).

Regression analyses indicated a weak but significant correlation between vaccine dose and GP-binding antibodies assessed by glycoprotein ELISA (left upper panel). A significant strong correlation between vaccine dose and titers of neutralizing antibody titers was also identified using the PsVNA assay (right upper panel). Such correlations were not observed using whole virions or infectious Ebolavirus (lower panels), which is likely to reflect the lower sensitivity and thus weaker discriminatory capacity of these assays. The size of the symbols reflects sample sizes.

B. Month 6

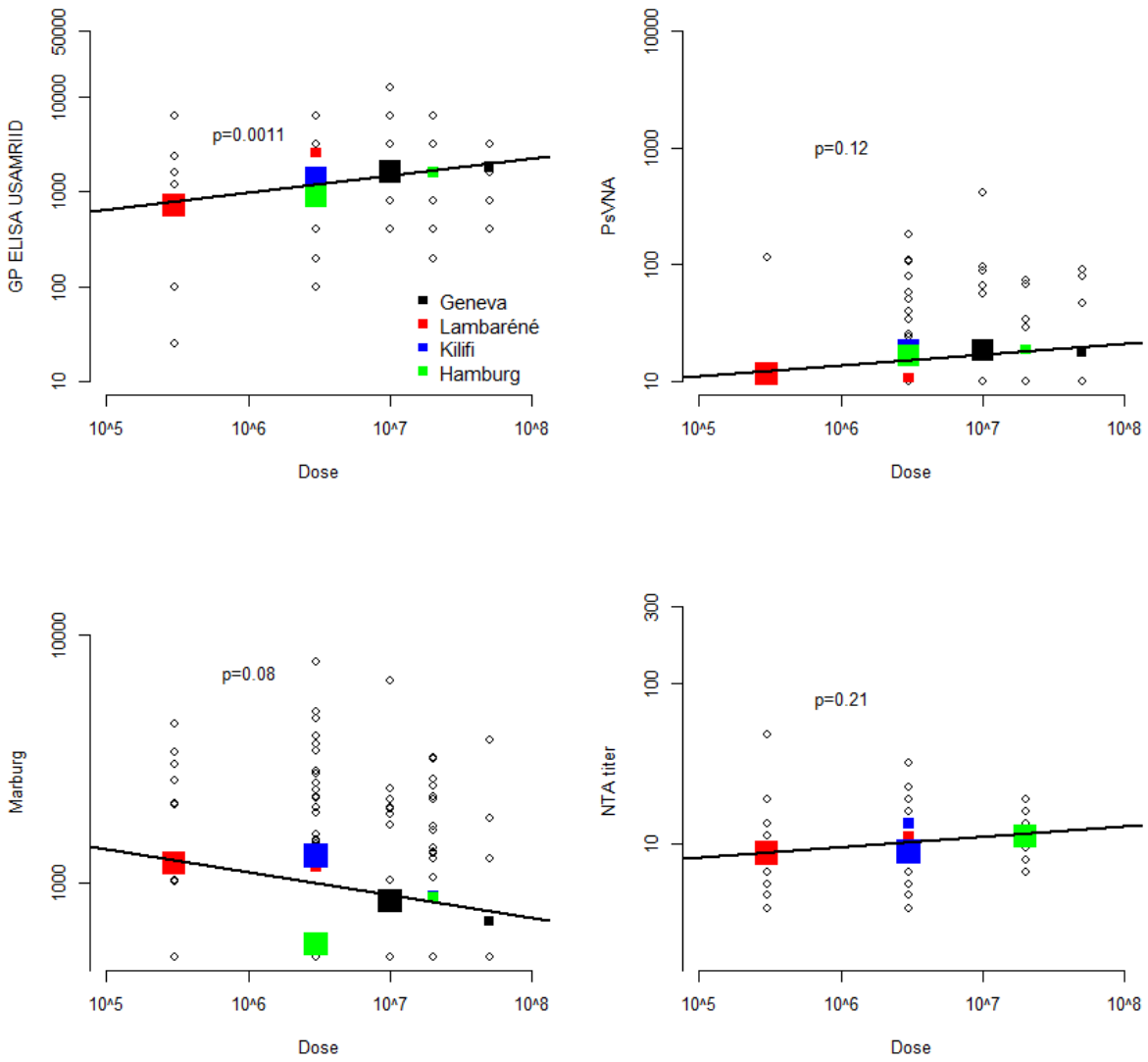


Figure S8B: Association between doses and month-6 GMT/GMC.

Method: linear regression with mixed effects on intercept (for potential center effect).

Regression analyses indicated a persisting significant correlation between vaccine dose and GP-binding antibodies (left upper panel). Such correlations were not observed using the other assays, which is likely to reflect the lower sensitivity and thus weaker discriminatory capacity of these assays at low or moderate titers. The size of the symbols reflects sample sizes.

Association between doses and seropositivity

Table S12: Associations between doses and seropositivity.

	Doses					
	$3x10^5$	**$3x10^6$**	**$1x10^7$**	**$2x10^7$**	**$5x10^7$**	**p-value**
Day 28						
GP ELISA USAMRIID	20 (100.0)	49 (100.0)	34 (100.0)	10 (100.0)	13 (100.0)	NA
PsVNA50	11 (55.0)	43 (87.8)	30 (80.2)	10 (100.0)	13 (100.0)	0.0006
Whole Virion ELISA	13 (65.0)	18 (36.7)	14 (41.2)	14 (46.7)	7 (50.0)	0.7719
NTA titer	14 (70.0)	37 (75.5)		20 (66.7)		0.7101
Day 180						
GP ELISA USAMRIID	15 (93.8)	46 (100.0)	33 (100.0)	30 (100.0)	15 (100.0)	0.1140
PsVNA50	15 (93.8)	10 (21.7)	9 (27.3)	4 (40.0)	4 (26.7)	0.1058
Whole Virion ELISA	9 (56.3)	23 (48.9)	11 (32.4)	13 (43.3)	4 (26.7)	0.0854
NTA titer	6 (37.5)	33 (70.2)		6 (60.0)		0.1320

Table S10: N and % of seropositive subjects according to dose. The p value was obtained using the Cochran-Armitage test for determining a trend across doses.

Association between doses and seroresponses

Table S13: Associations between doses and seroresponses.

	Doses					
	$3x10^5$	**$3x10^6$**	**$1x10^7$**	**$2x10^7$**	**$5x10^7$**	**p-value**
Day 28						
GP ELISA USAMRIID	17 (85.0)	47 (95.9)	33 (97.1)	10 (100.0)	13 (100.0)	0.0477
PsVNA50	10 (50.0)	36 (73.5)	24 (70.6)	10 (100.0)	13 (100.0)	0.0008
Whole Virion ELISA	10 (52.6)	12 (24.5)	6 (17.6)	8 (28.6)	2 (14.3)	0.0594
NTA titer	8 (42.1)	15 (30.6)		13 (43.3)		0.7734
Day 180						
GP ELISA USAMRIID	14 (87.5)	45 (97.8)	33 (100.0)	10 (100.0)	13 (100.0)	0.0462
PsVNA50	1 (6.2)	7 (15.2)	9 (27.3)	2 (20.0)	3 (23.1)	0.1617
Whole Virion ELISA	5 (33.3)	11 (23.4)	5 (14.7)	4 (14.3)	0 (0.0)	0.0108
NTA titer	2 (13.3)	12 (25.5)		3 (30.0)		0.3011

Table S11: N and % of seroresponders according to dose. The p value was obtained using the Cochran-Armitage test for determining a trend across doses.

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